Hsp90 binds to and promotes the clearance of tau, which is thought to reduce the formation of neurotoxic aggregates. Tau is an intrinsically disordered protein and it is unclear what role, if any, Hsp90 has in controlling its structure and dynamics. Hsp90 cooperates with numerous co-chaperones such as the immunophilin FKBP51, which assists in regulating the folding and processing of client proteins like tau. Defining the precise interactions between tau and the Hsp90 chaperone network is important for understanding the role of tau in Alzheimer's Disease. In this study, nuclear magnetic resonance (NMR) spectroscopy was used to probe the interaction between 15N-labeled tau, Hsp90 and FKBP51. The results demonstrate that two hydrophobic hexapeptide motifs located at residues 275-280 and 306-311 in tau's C-terminus bind to Hsp90 and FKBP51. This was determined by observing a significant reduction in the intensity ratios of HSQC spectra for free tau and tau in complex with Hsp90 and FKBP51. Resonances that show reduced intensities in the absence of line broadening are probably undergoing chemical exchange with a bound conformation. Several residues near the N-terminus of the protein also show a similar reduction in intensity upon addition of Hsp90 and FKBP51. Formation of the ternary complex around the client protein tau is congruent with currently proposed models suggesting that the binding of FKBP51 and Hsp90 assist in tau regulation, thereby triggering its recycling back to the MT surface.

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Single Molecule AFM Force Spectroscopy Analysis of Alpha-Synuclein Misfolding

Alexey V. Krasnoslobodtsev[1], Jie Peng[2], Ivan Volkov[3], Jean-Cristophe Rochet[4], Yuri Lyubchenko[1].
[1]University of Nebraska Medical Center, Omaha, NE, USA, [2]Shanghai Jiao Tong University, Shanghai, China, [3]Saint Petersburg State University, Saint Petersburg, Russian Federation, [4]Purdue University, West Lafayette, IN, USA.

Protein misfolding is a transient state during self-assembly into aggregates defining the molecular mechanism of the development of Alzheimer's, Parkinson's and other neurodegenerative diseases. Misfolding and aggregation of alpha-synuclein (α-Syn) is tightly linked to the development of Parkinson's disease. Here we applied single molecule AFM force spectroscopy (SMFS) to probe transient misfolded states of α-Syn measuring pair-wise interactions between individual α-Syn molecules at conditions that induce conformational transitions associated with enhanced aggregation. In the SMFS approach we probed the interactions between α-Syn covalently attached to the AFM probe and substrate by the C-terminal cysteine. We show that at conditions close to physiological, addition of spermidine results in dramatic increase of the protein's propensity to misfold. Additionally, using SMFS we detected and characterized misfolded dimers of α-Syn, the simplest aggregated form of α-Syn. Our results demonstrate that more than one segment within the protein molecule is responsible for the initial association of α-Syn into dimers and potentially into higher-order oligomers and fibrils. This finding suggests that even the first step of α-Syn self-assembly (dimerization) possesses a certain degree of heterogeneity. We hypothesize that these different misfolded conformations can lead to different types of oligomers and define the aggregation pathway. The marked differences in the misfolding patterns between WT α-Syn and single point mutants might be responsible for the higher propensity of the mutants to aggregate and cause early-onset PD.

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Macromolecular Crowding Stabilizes the Functional, Non-Toxic State of IAPP by Suppressing its Fibrillation

Janine Seeliger, Alexander Werkmüller, Roland Winter.
TU Dortmund, Dortmund, Germany.

The interior of the biological cell is known to be a crowded milieu, which significantly influences protein association and aggregation. As several cell degenerative diseases, such as Parkinsons disease or type-2 diabetes mellitus, are related to the misfolding, self-association and subsequent fibrillation of amyloidogenic peptides, understanding of the impact of macromolecular crowding on these processes is of high biomedical importance. This study focuses on the properties of human islet amyloid polypeptide (hIAPP) in crowded environments of two different kinds: network-like structures formed by polysaccharides and high concentrations of inert globular proteins. Two distinct processes could be distinguished in these crowded solutions: The formation of stable globular off-pathway species, and the usual hIAPP aggregation pathway from a disordered monomeric structure via nuclei formation to fibril formation. To which extent the different pathways are populated is shown to depend markedly on the crowder concentration and the geometry of the confinement. Different to other amyloidogenic peptides, the latter process is retarded or even inhibited at high crowding concentrations, but unchanged on the mechanistic level. As hIAPP is related to type-2 diabetes mellitus and presumably responsible for the disease accompanying β-cell-membrane permeabilization and the final β-cell loss, hIAPP specific cytotoxicity assays were conducted as well. Conversely to the high cytotoxicity exhibited by the normal fibrillation pathway, the data reveal a non-toxic effect for the off-pathway species stabilized through the crowding agents. From these results it can be postulated that cellular crowding is able to stabilize the native, non-toxic and functional conformation of hIAPP inside the biological cell.

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Surfactant Properties and Interface Induced Aggregation of Tau Proteins

Alexandra Hyler, Ayan Ray, Brandon Ricke, Benjamin Combs, T. Christopher Gamblin, **Prajnaparamita Dhar**.
University of Kansas, Lawrence, KS, USA.

Abnormal aggregation of microtubule associating protein, tau, into neurofibrillar aggregates , due to protein mutations, is a defining hallmark of several neurological diseases. Recent research indicates that the polymerization of soluble tau proteins into paired helical filaments may be influenced by the hydrophobic properties of its monomers, the presence of inducers and the local environment. In this work, we will discuss our results from using five HTau 40 protein variations: wild type (WT), pseudophosphorylated (7-phos), mutations on the binding domain (P301L), assembly incompetent protein (I277/308P), and mutations on the N terminal (R5L), to study template induced adsorption and aggregation of Tau proteins at a model hydrophobic interface.

Traditional biophysical techniques such as surface pressure vs. time (adsorption isotherms) are used to record adsorption kinetics. We find that even though tau is a soluble protein, it is highly surface active at nanomolar concentrations and demonstrate a two-step adsorption to the hydrophobic interface. Further, the adsorption kinetics is dependent both on the concentration and protein mutation. However, almost all the proteins studied here demonstrate a saturation concentration of a few hundred nanomoles, which is much lower than the bulk concentration where protein aggregation is recorded. Using an active microrheology technique unique to our lab, we also find that surface viscosity of the adsorbed protein films increase by orders of magnitude with time, indicating protein-protein interactions. However, the kinetics of this increase depends on the mutations on the protein. Further, TEM images of the protein solution obtained from the surface indicate the formation of protein oligomers. In summary, our results indicate that the soluble Tau proteins have interesting surfactant properties even at nanomolar concentrations that may play a role in their aggregation during Alzheimer's disease.

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Coarse Grain Simulations Providing a Unifying Framework for Explaining Polyglutamine Aggregation Mechanism

Siddique Khan, Nicholas Lyle, Rohit V. Pappu.
Washinton University in St. Louis, St. Louis, MO, USA.

Experiments and atomistic simulations show that homopolymeric polyglutamine forms heterogeneous distributions of collapsed, globular conformations in aqueous solutions. Atomistic simulations of monomer-dimer equilibria show that disordered polyglutamine globules associate to form disordered dimers, characterized by interactions between surface residues (the docked state) and interpenetrating chain molecules (the entangled state). Suppression of conformational fluctuations destabilizes the entangled state and inhibits dimerization. Similarly, naturally occurring flanking sequences from huntingtin destabilize the entangled state vis-à -vis the docked state.

Our coarse-grained simulations help to understand the impact of the relative and absolute stabilities of entangled and docked states on the aggregation processes. A phenomenological pair potential is used to model the interplay between these states. Results from our coarse-grained Langevin dynamics simulations are summarized as follows: We define pairwise energy scale ΔU as (Ue - Ud) representing the energy gap between the entangled and docked states, reference state being the bistable situation of ΔU = 0 with Ud = Ue = 4kT, describing the association of homopolymeric polyglutamine molecules. Fixing Ue and increasing ΔU by increasing docked state stability, leads to an increase in the rate of monomer loss and formation of small number of large disordered clusters vis-à -vis the reference bistable state, describing modulation effects of the N-terminal flanking sequence from huntingtin. Conversely, increasing ΔU by destabilizing the entangled state decreases the rate of monomer loss vis-a-vis the reference bistable state accompanied by the formation of large, ordered clusters, describing the effects of C-terminal